Exhibit E-2

effects upon the vagina than did Gynemesh PS, [12-14] the present study observed a higher ratio of M2 to M1 phenotype (macrophage polarization) and increased anti-inflammatory cytokine IL-10 in the lighter but not in the heavier mesh implanted vagina. Similar findings of improved material integration and remodeling associated with increased M2 macrophage populations have been observed in a number of other studies such as those in cardiac, dermal, and orthopedic applications of implantable materials of both biologic and synthetic origin. [18,22-25] In a recent study, [18] fifteen biologically derived surgical meshes were examined for both histologic outcomes and macrophage polarization profile at 14 and 35 days post-implantation in a partial thickness rodent abdominal wall defect model. The study showed that the number of M2 cells and the M2:M1 ratio at 14 days post-implantation were strongly correlated with semi-quantitative scoring of the histomorphologic appearance of the site of implantation at 14 days and were also predictive of the downstream histologic outcome at 35 days post-implantation. Taken together, this suggests that materials which elicit a higher percentage of M2 cells at the tissue interface may be associated with improved tissue integration and fewer complications in the long term.

There were a number of limitations of the present study. First, only one time point was examined, representing a cross sectional snapshot of a highly dynamic inflammatory process. It should also be noted that, due to the presence of an absorbable component poliglecaprolactone 25), the mesh burden associated with the UltraPro mesh and the local composition of the material is also dynamic. Thus, the host response to UltraPro may have a transient component which is not present in the other mesh materials. While statistically significant differences were observed between materials at 90 days, the magnitude of these differences was relatively small. Evaluation of macrophage phenotype at earlier times may have yielded larger differences, but is

likely not possible in a primate model due to cost and ethical considerations. Second, only one marker of M1 and M2 macrophage phenotypes was used in the present study. It is well known that macrophage phenotype occurs along a spectrum between M1 and M2 with multiple intermediate phenotypes.[21] While this represents the first such attempt to measure macrophage polarization in response to material implantation within the vagina, future studies are needed to better define both the phenotype and the function of the cells participating in the host response to implanted mesh to better understand their impact upon tissue integration versus degradation and the occurrence of complications in the long term.[34,35] Third, the present study describes a macrophage centered approach to the evaluation of the host response at the mesh-tissue interface. Future analyses could specifically evaluate the inflammatory reaction as a function of distance from the mesh surface or within pore spaces. This may be particularly important given that additional cell types, including a notable presence of T-lymphocytes, was observed with increasing distance from the mesh surface. Lastly, only mesh introduced by sacrocolpopexy was examined in the present study. Future studies should examine whether there are differences in the host response between mesh introduced by sacrocolpopexy versus transvaginally, and attempt to correlate the findings to the differences in rates of complications which have been observed for these two procedures.

In conclusion, the host response to polypropylene mesh consists predominantly of macrophages polarized to a pro-inflammatory M1 phenotype at 12 weeks post-surgery. However, implantation of lighter weight, higher porosity mesh generally attenuated the pro-inflammatory M1 response. These findings correlate with those of a previous study demonstrating that lighter weight, higher porosity mesh was also associated with fewer negative effects upon vaginal tissue quality. This suggests that the chronic M1 pro-inflammatory

response to mesh may drive tissue degradation eventually leading to mesh exposures over time similar to what is observed clinically; however, additional work is required to establish a causal relationship. An improved scientific understanding of the mechanisms of the host response to synthetic mesh materials placed in the vagina has the potential to significantly affect the design of next generation mesh materials, inform clinical practices and improve outcomes in pelvic floor repair.

CLINICAL IMPLICATIONS

- Regardless of mesh type or textile properties, the host response to mesh consists of predominantly of M1 pro-inflammatory macrophages.
- Implantation of lighter weight, higher porosity mesh generally attenuated the pro-inflammatory response, suggesting a link between mesh burden and the host inflammatory response.
- These findings correlate with those of a previous study demonstrating that lighter weight, higher porosity mesh was also associated with fewer negative effects upon vaginal tissue quality.
- This suggests that the chronic M1 pro-inflammatory response to mesh may drive tissue degradation eventually leading to mesh exposures over time similar to what is observed clinically.

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TABLES

Table 1. Mechanical and structural characteristics associated with each mesh.

	Gynemesh (Ethicon)	UltraPro (Ethicon)	Restorelle (Coloplast)
Weight (g/m2)	44	31	19
Pore Size (µm)	2240	4000+*	2370
Porosity (%)	64±2.1	69 ± 1.8	78 ± 3.0
Stiffness (N/mm)	28±2.7	22±2.8	11±0.89

*UltraPro contained a resorbable component (poliglecaprolactone 25) in addition to polypropylene allowing it to have very large pores (4mm) when this component is resorbed. Values reported with resorbable component dissolved.
Adapted from references #12 and 26.

Table 2. Demographic data collected (age, weight, gravidity and parity).

Groups	Age, y[a]	Parity[b]	Weight, kg[a]	POP-Q stage[b]
Sham	12.6 ± 2.8	3 (2, 6)	*7.3 ± 1.4	0 (0, 1)
Gynemesh	12.9 ± 2.2	4 (3.8, 5)	8.2 ± 1.6	0 (0, 0)
UltraPro	13.0 ± 2.2	3.5 (2, 5.8)	7.8 ± 1.4	0 (0, 0.25)
Restorelle	13.8 ± 1.7	5 (3, 5.5)	*10.0 ± 2.8	0.5 (0, 1.3)
P value[c]	0.780	0.970	0.042	0.700

[a] Mean ± SD, [b] Median (first quartile, second quartile), [c] Comparison of overall P value among groups, * Denotes statistical significance between groups (P<0.05)

Table 3. Total number of cells and percent surface marker postive cells in a 20X field.

Treatment (Gynemesh)	Total Number of Cells (per 20X field)	% of Positive Cells (per 20X field)
CD45	514±121	21.4±5.4
CD68	510±108	[c]10.5±3.9
CD3	510±114	[d]7.3±1.7
CD20	509±109	3.0±1.2
CD117	508±121	0.2±0.2
P value	1.00[a]	<0.001[b]

[a] Comparison of p-value among groups, significant difference if $p<0.05$; b Comparison between CD68, CD3, CD20, and CD117, [c] Significance seen between CD68 and CD20 and CD68 and CD117; [d] Significance seen between CD3 and CD20 and CD3 and CD117.

Table 4. total number of cells, percent of positive cells and ratio of m2/m1 macrophages seen in a 20X field (fiber)

Total Number of Cells, Percent of Positive Cells and Ratio of M2/M1 Macrophages in a 20x Field (Fiber)				
Treatment	**Total Number of Cells**	**% of M1 Positive Cells**	**% of M2 Positive Cells**	**Ratio M2/M1**
Sham	696±370	[b]0.8±0.7	[b]0.1±0.0	-
Gynemesh	642±215	6.8±2.8	3.5±2.2	[c]0.52±0.14
UltraPro	768±232	7.3±2.6	4.6±1.5	0.66±0.08
Restorelle	610±291	7.0±3.7	4.6±2.1	0.67±0.09
P value[a]	0.700	<0.001	<0.001	<0.001

[a] Comparison of p-value among groups, significant difference if $p<0.05$; [b] Significance seen between Sham and Gynemesh, Sham and UltraPro and Sham and Restorelle; [c] Significance seen between Gynemesh and UltraPro and Gynemesh and Restorelle

Table 5. Total number of cells, percent of positive cells and ratio of m2/m1 macrophages seen in a 20X field (knot)

Total Number of Cells, Percent of Positive Cells and Ratio of M2/M1 Macrophages in a 20x Field (Knot)				
Treatment	**Total Number of Cells**	**% of M1 Positive Cells**	**% of M2 Positive Cells**	**Ratio M2/M1**
Sham	679±327	[a]0.1±0.1	[a]0.1±0.0	-
Gynemesh	630±230	8.3±4.7	4.4±2.3	0.57±0.11
UltraPro	722±214	9.2±2.8	5.3±1.0	0.61±0.18
Restorelle	575±104	8.4±2.6	4.9±1.8	0.60±0.11
P value[e]	0.620	<0.001	<0.001	<0.001

[a] Comparison of p-value among groups, significant difference if $p<0.05$; [a] Significance seen between Sham and Gynemesh, Sham and UltraPro and Sham and Restorelle

Table 6. Correlation between percentage of positive cells and mesh area in a 20X image

Correlation Between Percentage of Positive Cells and Mesh Area in a 20X Image				
	% M1 Cells vs. Area		**% M2 Cells vs. Area**	
	Correlation Coefficient	**P value**	**Correlation Coefficient**	**P value**
Gynemesh	0.39	0.006	0.44	0.002
UltraPro	0.35	0.015	0.23	0.113
Restorelle	0.24	0.098	0.22	0.136
All Mesh	0.30	0.001	0.23	0.006

Table 7. Individual and ratio values of anti- and pro-inflammatory cytokines

Individual and Ratio Values of Anti- and Pro-inflammatory Cytokines					
Groups	**IL-10**[a]	**IL-4**[a]	**TNF-α**[a]	**IL-12p70**[a]	**(IL-10+IL-4)/ (TNF-α+IL-12p70)**[b]
Sham	1.30±0.31	0.33±0.01	0.38±0.11	0.26±0.06	2.58±0.52
Gynemesh	1.03±0.20	0.29±0.08	0.33±0.12	0.27±0.05	*2.17±0.78
UltraPro	1.12±0.22	0.27±0.11	0.28±0.15	0.23±0.07	3.01±0.90
Restorelle	1.27±0.22	0.263±0.05	0.26±0.05	0.30±0.15	*3.36±0.60
P value[c]	0.014	0.358	0.084	0.386	0.004

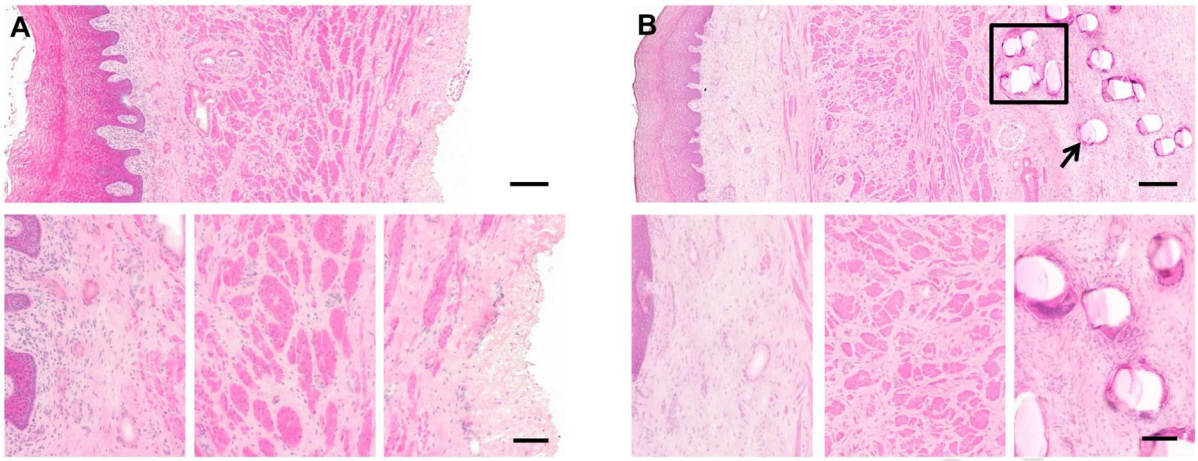
[a] pg/µg total protein, mean ± standard deviation; [b] unitless, mean ± standard deviation; [c] comparison of overall P value among groups, * Denotes statistical significance between groups ($P<0.05$)

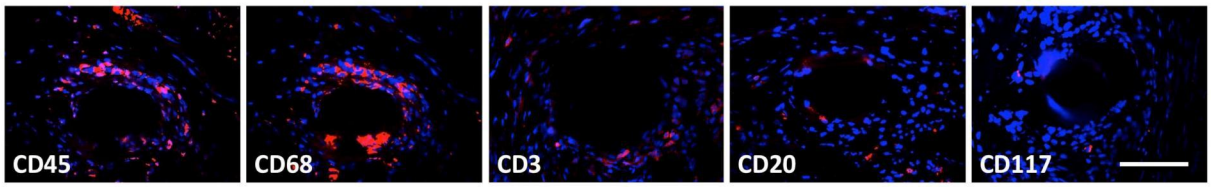
FIGURE LEGENDS

Figure 1: Representative histologic section (hematoxylin and eosin) taken from Sham (A) and Gynemesh (B) groups. The top panel contains a full view of the histologic section at 10X magnification (scale bar = 250 μm). Bottom panel shows higher magnification images of the subepithelial connective tissues, muscularis layer and the adventitia (A) or mesh-tissue interface (B) (20x magnification, scale bar = 100 μm). The histomorphologic appearance of the response to Gynemesh was characteristic of the response observed in all mesh implanted groups. A dense population of mononuclear and multinucleated giant cells can be observed at the mesh-tissue interface, decreasing in number with increasing distance from the mesh surface. Box in (B) indicates a mesh knot, and arrow indicates a mesh fiber.

Figure 2: Immunofluorescent labeling of cells participating in the host response to implanted mesh. Antibodies for CD45 (pan-leukocyte), CD68 (macrophage), CD3 (T lymphocyte), CD20 (B lymphocyte), and CD117 (mast cell) markers were used (red). DAPI (blue) was used to label nuclei. Positively labeled cells were predominantly located at the mesh surface, with fewer cells with increasing distance. All images at 40X magnification, scale bar = 100 μm.

Figure 3: Immunofluorescent labeling with antibodies to CD68 (Pan-macrophage, red), CD86 (M1 macrophage, orange), CD206 (M2 macrophage, green) and DAPI (nuclei, blue) is shown. Few positive cells were observed in sham-operated animals (not shown). Predominance of the M1 macrophage response was observed in Gynemesh PS, UltraPro and Restorelle groups. Combined fluorescent channels are shown in the top panel, and individual channels in the bottom panel. All images at 20X magnification, scale bars = 100 μm.





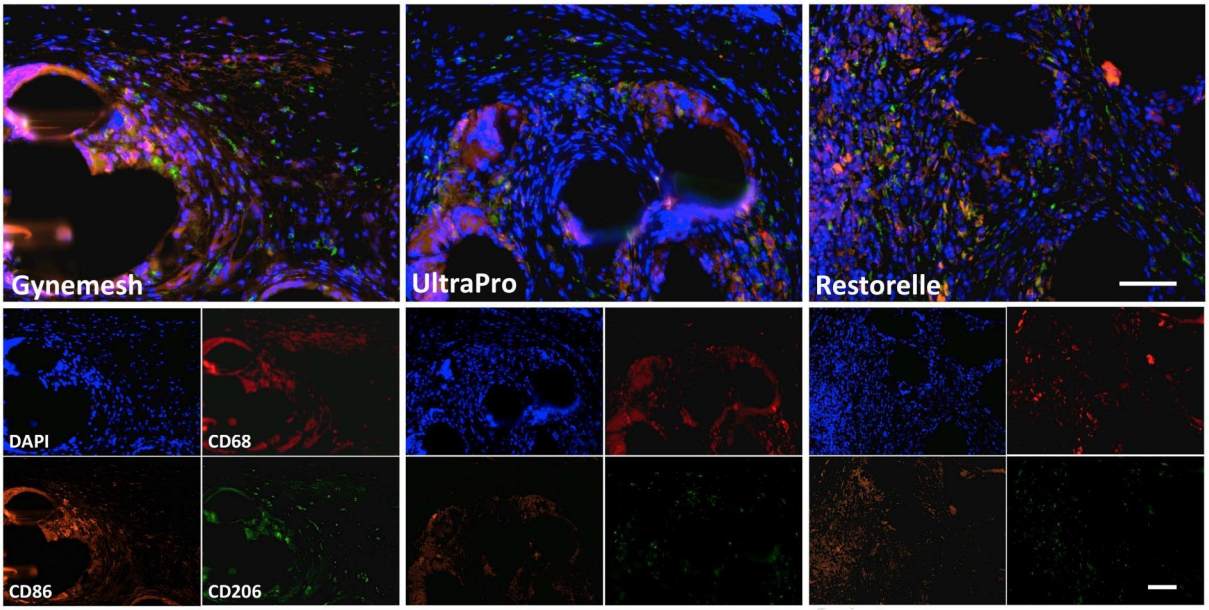


Table III: Antibodies used in immunofluorescence labeling

Primary Antibody				
Name	**Dilution**	**Catalog Number**	**Clonality**	**Company**
Rabbit Anti-CD45	1:600	ab10558	Polyclonal	abcam
Mouse Anti-CD68	1:100	ab955	Monoclonal	abcam
Rabbit Anti-CD3	1:50	A0452	Polyclonal	DAKO
Rabbit Anti-CD20	1:50	ab27093	Polyclonal	abcam
Rabbit Anti-CD117	1:50	ab32363	Monoclonal	abcam
Rabbit Anti-CD86	1:150	ab53004	Monoclonal	abcam
Goat Anti-CD206	1:150	sc-34577	Polyclonal	Santa Cruz
Secondary Antibody				
Name	**Dilution**	**Catalog Number**	**Wavelength**	**Company**
Alexa 594 Donkey anti-Mouse	1:100	A21203	590/617	Invitrogen
Alexa 568 Donkey anti- Rabbit	1:50 (CD3, CD20 & CD117) 1:200 (CD45)	A10042	578/603	Invitrogen
Alexa 488 Donkey anti- Goat	1:250 (CD206)	A11055	488/519	Invitrogen
Alexa 647 Donkey anti- Rabbit	1:250 (CD86)	A31573	650/668	Invitrogen